Commentary

A compelling genetic hypothesis for a complex disease: *PRODH2*/*DGCR6* variation leads to schizophrenia susceptibility

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The human genome sequence is facilitating the task of identifying disease genes, understanding their normal functions and why compromising their function is associated with specific features of a disorder and its inheritance pattern (1). Genetic disorders with simple dominant and recessive (Mendelian) patterns of inheritance are invariably caused by rare single-gene mutations that are both necessary and sufficient for the disease to manifest. However, some single-gene disorders can display a complex pattern of inheritance. The prototype for this class is fragile X syndrome (2), whose non-Mendelian and complex pattern of X-linked inheritance arises from dynamic evolution of the mutation within families (3). Thus, complexity in inheritance patterns is not inconsistent with single-gene defects although geneticists routinely equate the non-Mendelian nature of a trait with inheritance at multiple genes (4). Genetic dissection of multigenic disorders is truly challenging yet possible (5), particularly with the human genome sequence in hand. In an outstanding piece of sleuthing, Maria Karayiorgou and colleagues (6) now show that genetic variation in proline dehydrogenase on human chromosome 22q11 is a likely and significant cause of schizophrenia.

Schizophrenia is a common and severe mental illness of thought, emotion, and behavior that affects about 1% of the general population. It is a devastating disorder, probably unique to humans, affecting not only the sufferers but also their families. The intense familiality of schizophrenia has been recognized for a long time and siblings of schizophrenics have a 10-fold elevated susceptibility to the phenotype (7). The familial nature of schizophrenia does not conform to simple dominant or recessive modes of inheritance and, consequently, identifying the underlying genes have proved difficult and disease pathophysiology is still in doubt. Over the past three decades, scores of psychiatrists and geneticists have grappled with identifying the genetic determinants of schizophrenia, now universally thought to reside at multiple genes (8). These genetic mapping studies have proved to be frustrating because no single chromosomal region appears paramount and genomic locations identified by one group have seldom been replicated by others. There are many possible reasons for this outcome, both biological and methodological, but heterogeneity in disease causation is thought to be the most likely cause.

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One chromosomal region that appears to be contributory to schizophrenia susceptibility is that on human chromosome 22q11 (9). Despite its inconsistent involvement across mapping studies, its importance is demonstrated by the finding that 25–31% of patients with microdeletions of chromosomal material at 22q11 met diagnostic criteria for schizophrenia and associated disorders. These microdeletions are rare in the general population but are 80 times more frequent in adult schizophrenics and 240 times elevated in childhood-onset schizophrenia. Although childhood onset of schizophrenia is rare, this marked elevation of microdeletion frequency is a terrific clue because it solidly implicates a genomic region, at least for some cases of schizophrenia etiology. The specific genes involved, however, remained a mystery, until the present study (6), because the deletions remove many genes (10).

The success of the Liu *et al.* (6) study clearly owes a great deal to the public availability of the human genome sequence (11). The authors undertook the systematic screening of all known nine genes in a 1.5 million-bp interval defining the schizophrenia "critical" interval (10). This screening involved first the identification of common sequence variation within and outside the known genes, called single nucleotide polymorphisms. Subsequently, to test whether any of the variants were schizophrenia "markers" the authors conducted two genetic tests: (*i*) Were the variants in increased frequency among schizophrenics as compared with controls? (*ii*) Were the variants transmitted by parents to their affected children in significantly greater frequency than expected? These genetic tests were conducted in three independent and well-documented samples of schizophrenia cases. These tests led to one inescapable conclusion: genetic variation in the 22q11 region increased schizophrenia risk by 2-fold or more.

Although the proof is not absolute, the data of Liu *et al.* (6) strongly suggest that genetic variation in a specific gene, *PRODH2,* is the likely cause of schizophrenia susceptibility. The first line of evidence comes from the observations that the strongest association, both by elevated frequency and transmission, of schizophrenia is with single nucleotide polymorphisms within this gene. The second piece of evidence is that most of the variants in *PRODH2* associated with disease are identical to sequences in the neighboring pseudogene. Third, in a small sample, carriers of variants associated with the disease show elevated plasma proline levels; it is difficult to know whether brain proline levels are similarly affected. As in all studies of complex disorders, caveats abound, but the evidence for *PRODH2* culpability in schizophrenia is excellent and is more than enough to deserve continued careful scrutiny. *PRODH2* encodes proline dehydrogenase, a mitochondrial enzyme that converts proline to Δ^1-pyrroline-5-carboxylate and is involved in transfer of redox potential across the mitochondrial membrane. The gene is widely expressed

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in many tissues, including in the brain, and its homozygous deficiency, hyperprolinemia, is an inborn error of metabolism. There is some indication that heterozygous deficiency of *PRODH2* may be a cause of isolated hyperprolinemia (12). Importantly, mice created to be homozygous for *PRODH2* deficiency demonstrate a deficit in prepulse inhibition (13), a measure of sensorimotor gating affected in schizophrenics. Finally, proline may serve as a direct modulator of glutamatergic activity in the brain. Thus, the evidence for candidacy for *PRODH2* deficiency in schizophrenia is considerable.

The study by Liu *et al.* (6) is compelling for a variety of reasons and is a wonderful example of the power of genetic studies in the human. This study has five features that are important to emphasize. First, there is incredible value to clinical genetic studies of apparently rare syndromes because they allow us to make cogent genetic hypothesis, such as deficiency of genetic material in 22q11 predispose to neuropsychiatric disease. Second, there is immense value to well-documented and carefully examined clinical cases and their families: without the multiple samples analyzed the faith in the results would have been diminished. Third, searching for the genetic effect in patients at higher risk, such as those with younger age-at-onset or childhood-onset schizophrenia, is always a good strategy. Fourth, an absolute requirement for genetic dissection of a complex disease should be the comprehensive screening of all genes in a region and the examination of many single nucleotide polymorphisms. Although this is considerable work, even with current genomics technology, Liu *et al.* (6) were rewarded by finding multiple markers associated with disease in two distinct tests. A low-resolution screen always fails to distinguish between the absence of an effect and the failure to detect it. Many previous marker association studies in schizophrenia were doomed because they examined only one single nucleotide polymorphism per gene. Fifth, the study of an animal model, even when it does not demonstrate a ''disease'' phenotype but a biochemical and neural phenotype, is indispensable (13).

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An emerging idea in human genetics is that of ''genomic disorders'' where the mutation results from the specific genomic structure, particularly duplications. This is particularly the case with human chromosome 22, which is extremely rich in segmental duplications (14). *PRODH2* is one example of such ''paralogous'' genes on this chromosome because it resides within a genomic segment that is duplicated; its paralog, *PRODH2-P*, is most likely a pseudogene (14). Extensive nucleotide similarity between these two genes is the reason for apparent gene conversion, rather the use of the pseudogene as a template to replicate *PRODH2* in some genomes. Thus, sequence variation in a pseudogene with no functional consequences can, on occasion, be copied into the functional gene and lead to disease (6). If proven, schizophrenia, at least the *PRODH2* variety, arises from the peculiarities of our genome sequence and is a genomic disorder. Thus, some of the complexities in inheritance could arise from this feature.

The recognition that sequence structure affects gene function and this schizophrenia study could not have been attempted in the absence of an accurate and finished reference genome sequence for human chromosome 22 (11). The finished sequence clarifies the gene content and thus allows the systematic gene search strategy. Thus, finishing the human genome sequence remains a top priority. This is particularly so in the regions that are difficult to finish because they contain a greater frequency of sequence repeats and duplications. Indeed, these regions may have a surfeit of complex disease genes.

Liu *et al.* (6) have raised as many questions as they have answered. Chief among them is the contribution of *PRODH2* to schizophrenia risk in the population. Is *PRODH2* deficiency necessary and sufficient for schizophrenia? Is this so in at least some families? Because the set of markers within *PRODH2* that increase disease risk is known, and these should be transmitted within families by Mendelian rules, is there a clustering of schizophrenia in the families examined? What other genes are involved? Finally, is brain elevation of proline a necessary step in schizophrenia?

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